Nitrous Oxide Emissions from Soil Profiles Seeded with Pulse Crops

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Abstract

Inoculation of legumes with *Rhizobium* spp. is a common worldwide agricultural practice that is used to increase crop yield and to improve soil fertility without adding nitrogen (N) fertilizers. There is concern that N_2 fixation by legumes enhances emissions of nitrous oxide (N_2O) – a powerful greenhouse gas (GHG). The objectives of this experiment were: 1) to measure N_2O emissions from soil profiles under inoculated and non-inoculated pulse crops; 2) to investigate the relationship between N_2O emissions and N_2 fixation by pulse crops. The experiment was carried out in the Phytotron of the University of Saskatchewan with soil flats seeded with inoculated and non-inoculated lentils and peas and fertilized spring wheat. N_2O emissions from soil profiles were measured using Profile Gas Samplers (PGS), and were analyzed with a GC. Results indicated that inoculated lentils and peas did not substantially increase N_2O emissions, and N_2O emissions may not be associated with N_2 fixation under the conditions in this experiment.

1 Introduction

Rhizobia can invade and form nodules on the roots of leguminous plants to fix nitrogen from the atmosphere. Inoculation of legumes with *Rhizobium* spp. is a common worldwide agricultural practice used to increase crop yield and to improve soil fertility without adding nitrogen fertilizers (Bohlool et al., 1992; Peoples and Craswell, 1992). Substantial acreages of pulse crops such as field pea and lentil are grown in Western Canada. Recently, questions have arisen about whether the cultivation of pulse crops enhances N loss in the form of N_2O – a powerful greenhouse gas (Svensson et al., 1991; Klemedtsson, et al., 1991; Aulakh et al., 1991; Arrese-Igor and Aparicio-Tejo, 1992; Kilian and Werner, 1996). The enhanced N_2O emissions from agricultural and natural ecosystems are believed to be caused by increasing soil N availability driven by increased fertilizer use, agricultural nitrogen (N_2) fixation, and N deposition (IPCC, 2001). The current methodology for estimating N_2O emissions on a national level considers N_2 fixation by legumes an important source of N_2O emissions (IPCC, 2001). However, the contribution of inoculated pulse crops to N_2O emissions under dryland conditions in

western Canadian is not well understood. The objectives of this experiment were: 1) to measure N_2O emissions from soil profiles seeded with inoculated and non-inoculated lentils and peas; 2) to investigate the relationship between N_2O emissions and N_2 fixation by lentils and field peas.

2 Materials and Methods

2.1 Soil and soil flats

A Chernozemic soil was collected from the Dark Brown soil zone and was homogeneously mixed with sand. The resultant soil has a texture of sandy loam with a bulk density of 1.35 g cm^{-3}, and total C, N and P contents of 34.4, 2.44, 0.68 g kg^{-1} soil, respectively. Using this soil, 16 soil flats (45 cm ∝ 50 cm ∝ 60 cm) were built for plant growth.

2.2 Inoculant preparation

Rhizobial inoculants were prepared by growing two *R. leguminosarum biovar viciae* isolates 99A1 and RGP2 in yeast extract-mannitol (YEM) medium. Cultures were placed on a G10 Gyrotory Shaker (at 22±C, 150 rpm) for 3 d prior to inoculation.

2.3 Plant inoculation, fertilization and growth conditions

Lentil (*Lens esculenta* Moench *cv. Milestone*) and pea (*Pisum sativum* L. *cv. Mozart*) were inoculated at a rate of 3 ml g^{-1} of 99A1 and RGP2, respectively, at sowing date. Non-nodulated pulse crops were fertilized with NH_4NO_3 (100 kg N ha^{-1}). Spring wheat (*AC Barrie*), used as a reference crop, was fertilized at a rate of 100 kg N ha^{-1}. P and K were applied at a rate of 15 kg P ha^{-1} and 35 kg K ha^{-1}, respectively. Each treatment was replicated 3 times.

Plants were grown in the phytotron of University of Saskatchewan (daytime: 22±C for 16 h, nighttime: 16±C for 8 h). Soil moisture was maintained at 60-80% of field capacity by monitoring with a time domain reflectometry (TDR) (Topp et al., 1996). Plants were sown on July 15, 2003. They emerged on July 22, and were harvested on October 23, 2003.

2.4 N_2O measurement

A multi-level Profile Gas Sampler (PGS) was constructed for gas sampling from soil profiles at different depths. Gas samples were taken once a week using a syringe and injected into evacuated tubes, and their N_2O concentrations were analyzed using a gas chromatograph (GC) (Varian CP3800). The GC consists of three electron capture detectors (ECD). The carrier gas was 95% argon and 5% methane, and make-up gas was helium. The temperatures for detector, oven and injector were 370±C, 35±C, and 100±C, respectively. The software for the integrator/recorder was Varian Star 5.5. During the GC operation, a gas sample of 300 νL was introduced into the injection system for the analysis of N_2O concentrations.

2.5 Other measurements

Nitrogen fixation by lentil and pea, as indicated by nitrogenase activities of nodules, was determined using the C_2H_2 reduction assay (ARA) (Hardy et al., 1968). Plants were carefully uprooted from the soil flats so that nodules were least removed and then were placed as quickly as possible in screw-cap jars (550 ml in volume) fitted with a rubber seal for gas injections and sampling. Then, 25 ml of air was drawn out of each sealed jar and replaced with the same volume of acetylene. After 30 min of incubation (under 25±C), about 0.5 ml gas sample was drawn from the jar using a syringe and was injected into a gas chromatography (Model 5790A Series, Hewlett-Packard) for C_2H_2 reduction analysis. Within the GC system, the gas carrier was dinitrogen gas (N_2) with a flow rate of 30 ml min^{-1}. The temperatures for oven, injector and detector were 40±C, 50±C and 150±C, respectively. The pressures for N_2 and H_2 and air were 160 kPa, 200 kPa, and 150 kPa, respectively.

Plant biomass was measured at different growing stages by randomly removing and drying (at 60±C for 48 h in oven) one plant from each soil box. Plant grain yields were measured at harvest time.

3 Results and Discussion

3.1 Plant growth and nitrogenase activities

Inoculation of *R. leguminosarum* 99A1 onto lentil and RGP2 onto field pea provided N for plant growth. Nitrogen fixation by nodules, as indicated by nitrogenase activities, was significantly higher in inoculated lentil and pea than in non-inoculated plants (Table 1). However, plant biomass and grain yields were generally not significantly different between inoculated and fertilized plants.

Table 1 Plant Growth and Nitrogenase Activity of Nodules

Plant	Inoculant /fertilizer	Shoot biomass (g $plant^{-1}$)	Root biomass (g $plant^{-1}$)	Yield (g m^{-2})	Nitrogenase activity (νmol g^{-1} h^{-1})
Lentil	99A1	5.12 ″ 1.59ab	0.07 ″ 0.04a	254.36 ″ 11.04b	14.06 ″ 1.41a
Lentil	100 kg N ha^{-1}	2.55 ″ 1.37b	0.07 ″ 0.01a	250.10 ″ 24.91b	4.29 ″ 1.36b
Pea	RGP2	6.60 ″ 0.93a	0.41 ″ 0.44a	298.59 ″ 25.85a	19.21 ″ 5.83a
Pea	100 kg N ha^{-1}	5.31 ″ 1.51a	0.09 ″ 0.03a	328.93 ″ 5.82a	2.25 ″ 0.38b

Nitrogenase activity and plant biomass were measured at the mid-stage of plant growth.
Values with different letter are significantly different ($p<0.05$).

3.2 N_2O production from soil profiles

Low N_2O production (0.3 -3.5 ppm) was observed 0-25 d after planting, after which N_2O concentrations leveled off and remained at the same level as the ambient atmosphere (Figs. 1-5).

Most of the N_2O production accumulated in the deeper layers (15-30 cm). This may have resulted from reduced gas exchange between the deeper layers and the surface and reduced aeration in the deeper layers, favoring denitrification.

The N_2O emissions from soil profiles showed the same patterns both for pulse crops and the cereal crop, and for inoculated and non-inoculated pea and lentil. Rhizobial strains 99A1 and RGP2 were not able to denitrify in pure cultures (Zhong et al., 2003). This indicated that inoculation of lentil and pea with these rhizobial strains did not enhance denitrification under the conditions of this experiment.

Nitrogen fixation by lentil and pea, as expressed by nitrogenase activities of nodules, was significantly increased in inoculated compared to non-inoculated legumes (Table 1). However, N_2O production from soil profiles was not significantly different between inoculated and non-inoculated lentil and pea (Figs. 1-4). In addition, a comparison of N_2O production from soil profiles between pulse crops (lentil and pea) and a cereal crop (spring wheat) indicated that the presence of pulse crops did not have an obvious impact on N_2O emissions from soil profiles (Figs. 1-5). These results suggest that N_2O emissions may not be associated with nitrogen fixation by pulse crops.

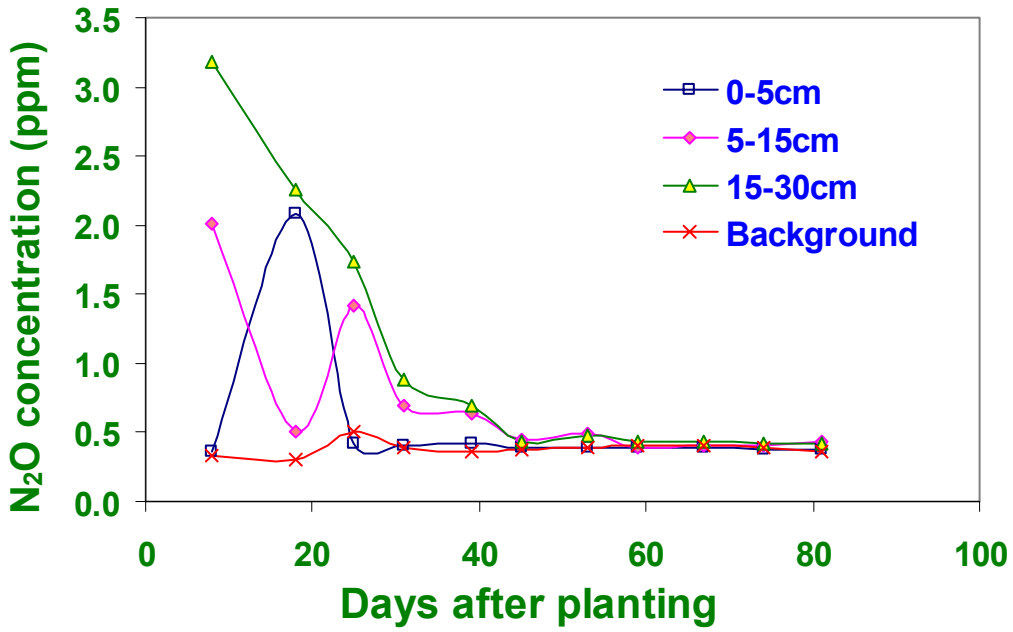

Fig. 1 N_2O concentrations in soil profiles of inoculated lentil

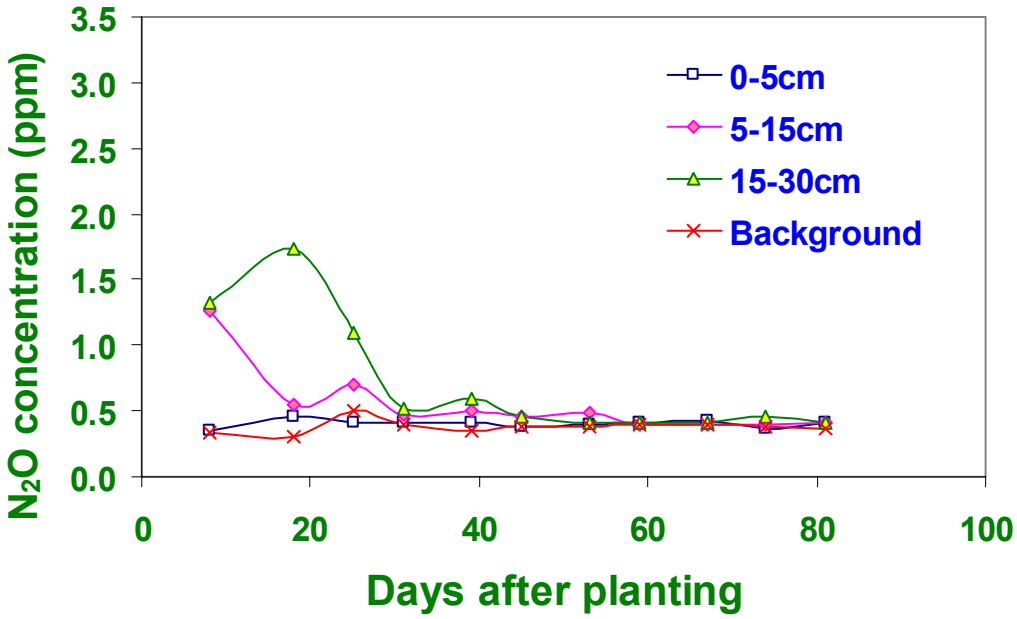

Fig. 2 N_2O concentrations in soil profiles of non-inoculated lentil

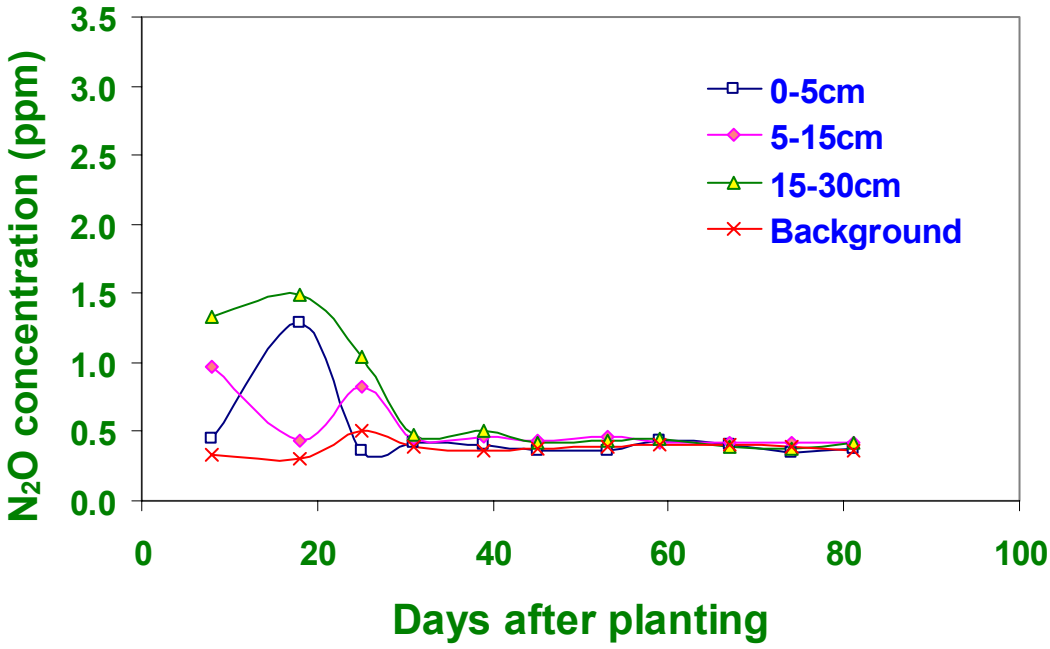

Fig. 3 N_2O concentrations in soil profiles of inoculated pea

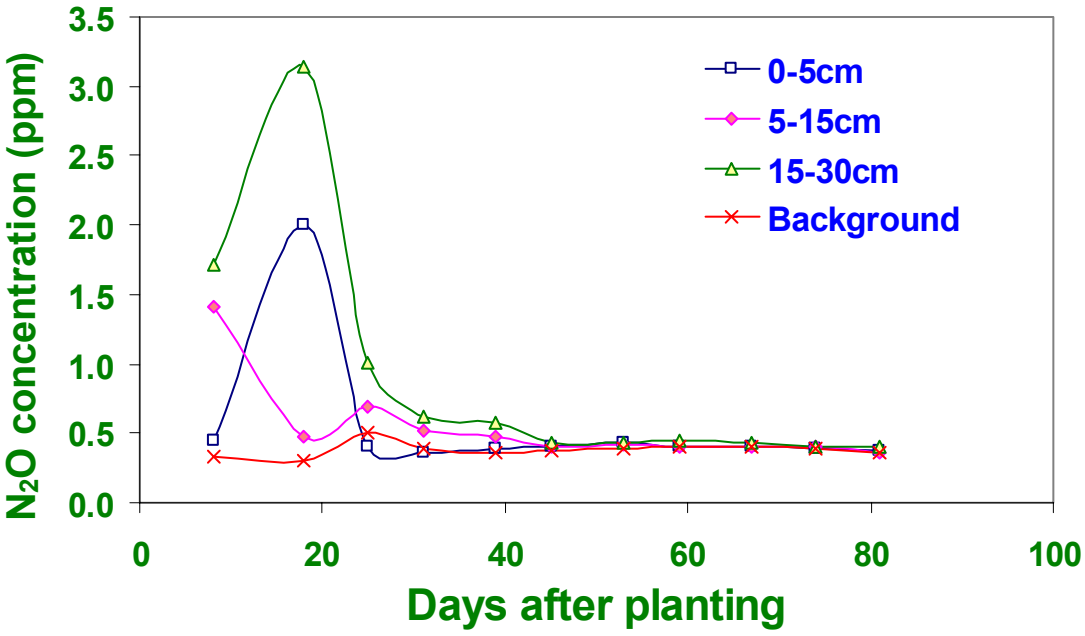

Fig. 4 N_2O concentrations in soil profiles of non-inoculated pea

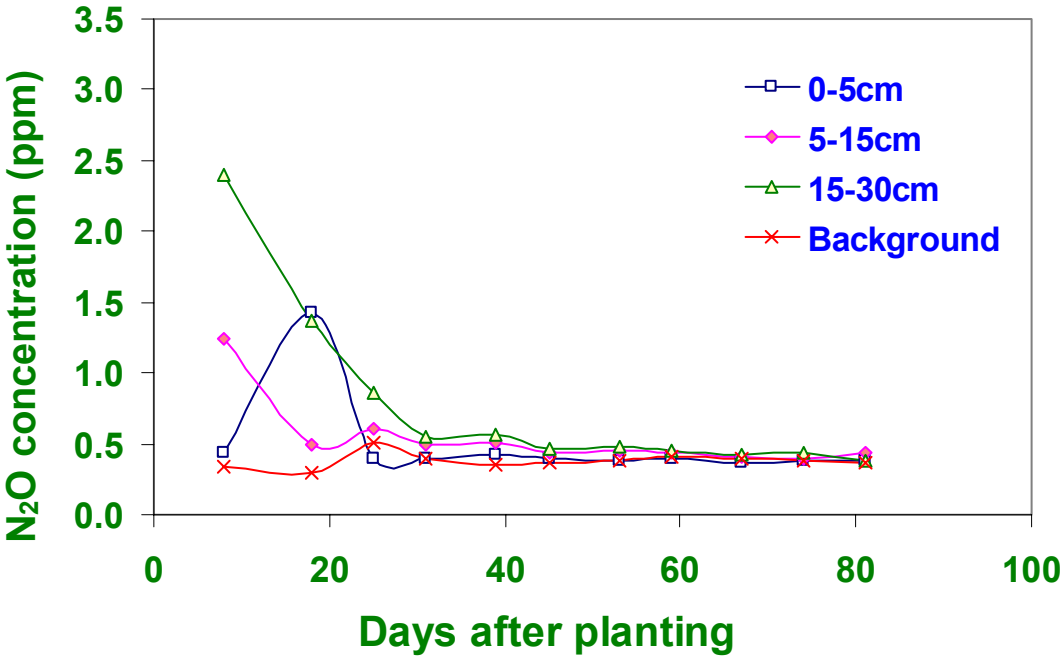

Fig. 5 N_2O concentrations in soil profiles of fertilized wheat

4 Conclusion

Inoculation of *R. leguminosarum* strains 99A1 and RGP2 onto lentil and pea stimulated plant growth, but N_2O production was not enhanced compared with fertilization with NH_4NO_3. Inoculating pulse crops with these two rhizobial strains increased soil fertility and crop yields, but did not increase N_2O production. This suggests that N_2O emissions may not be associated with nitrogen fixation by pulse crops under these conditions.

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